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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Judes Poirier	Art Unit:	1648
Serial No.:	09/500,162	Examiner:	Parkin, Jeffrey S.
Filed:	February 8, 2000		
Title:	PHARMACOGENETIC METHODS FOR USE IN THE TREATMENT OF NERVOUS SYSTEM DISEASES		

Commissioner of Patents
Washington, D.C. 20231

DECLARATION OF JUDES POIRIER, PH.D. UNDER 37 C.F.R. §§ 1.131 AND 1.132

1. I am the inventor of the above-referenced patent application.

2. I have read and understood the specification of U.S. Serial No. 09/500,162 (hereafter, "the application") and the Examiner's Office Action, issued on October 31, 2002, in connection with the above-referenced case.

3. The following data are presented to overcome the rejection of claims 1 and 3-15 under 35 U.S.C. § 112, first paragraph, for lack of enablement.

4. My discovery, that the *apoE4* allele in patients with AD and non-AD neurological diseases is associated with poor patient outcome or response to therapy, is further supported in the body of work published by several groups following the filing of the application. These publications, summarized below, describe clinical studies conducted by several researchers.

5. Clinical studies conducted by Fazekas and colleagues (J. Neurol. Neurosurg. Psychiatry, 69:25-28, 2000) on 83 patients diagnosed with multiple sclerosis demonstrated a positive correlation between the presence of at least one *apoE4* allele with increased tissue destruction or decreased repair (page 25, abstract of Fazekas *et al.*). The study specifically concluded that the course of multiple sclerosis is negatively impacted by the presence of at least one *apoE4* allele.

6. Leung *et al.* (Stroke, 33:548-552, 2002) demonstrate a relationship between outcome in 72 patients with aneurysmal subarachnoid hemorrhage (SAH) and the *apoE4* genotype. The prospective study was performed on all patients admitted with spontaneous aneurysmal SAH over the course of a two-year period. Leung *et al.* observed that *apoE4* carriers were more susceptible to an unfavorable outcome, regardless of therapeutic treatment (see Leung *et al.*, pages 549-550).

7. Drory *et al.* (J. Neurol. Sci., 190:17-20, 2001) investigated the association of *apoE4* allele load and amyotrophic lateral sclerosis (ALS). Results from this study demonstrated that in 100 patients diagnosed with ALS, relative to 133 healthy subjects, those patients carrying at least one copy of the *apoE4* allele showed poorer prognosis with respect to survival and disease progression (see Drory *et al.*, abstract, p. 17). Furthermore, Drory *et al.* reiterate that the *apoE4* allele has also been associated with poor clinical outcome for Alzheimer's Disease, dementia associated with stroke, and multiple sclerosis (see Drory *et al.*, p. 19).

8. In each of the foregoing clinical studies, the *apoE4* allele load was determined on diagnosed neuropathies. These studies demonstrate that carriers of the *apoE4* allele having a diagnosed neurological disease showed poorer prognosis than patients that do not carry the *apoE4* allele.

9. I analyzed 59 patients suffering from Parkinson's disease (PD) to determine if there is a relationship between a patient's *apoE* genotype and prognostic outcome. The study group was composed of Caucasian males diagnosed as suffering from PD and currently under

treatment with levodopa-carbidopa (SINEMET™). The PD patients in the study had an *apoE* allele distribution similar to a much larger randomized North American-population (see **Table 1, Exhibit A**). The PD patients were not suffering from any other central nervous system disease. To determine the PD patient's *apoE* genotype, 5 mLs of whole blood was drawn from each PD patient and used as a source of genetic material for *apoE* allele determination.

10. When I compared the PD patient's *apoE* genotype to their prognostic outcome by measuring improvement of symptoms of tremor and rigidity, I observed a strong negative correlation between the PD patient's prognostic outcome and their *apoE4* allele load by these parameters. PD Patients with no *apoE4* allele showed a good improvement, while those PD patient's with an *apoE4* allele showed a poor outcome (see **Table 2, Exhibit B**). Thus, I confirmed that a direct link exists between carrying an *apoE4* allele and the prognostic outcome of a patient with PD.

11. I analyzed 65 patients suffering from multiple sclerosis (MS) to determine if there is a relationship between a patient's *apoE* genotype and prognostic outcome. The study group was composed of Caucasian females diagnosed as suffering from MS and currently under treatment with interferon β-1B (BETASERON™). The MS patients in the study had an *apoE* allele distribution similar to a much larger randomized North American-population (see **Table 3, Exhibit C**). To determine the MS patient's *apoE* genotype, 5 mLs of whole blood was drawn from each MS patient and used as a source of genetic material for *apoE* allele determination.

12. When I compared the MS patient's *apoE* genotype to their response to drug treatment, which was designed to reduce symptomatic exacerbation of the disease, I observed that non-*apoE4* MS patients demonstrated a good prognostic outcome, while those MS patients that carried an *apoE4* allele demonstrated a poor prognostic outcome (see **Table 4, Exhibit D**). When examined solely on the presence or absence of an *apoE4* allele, the correlation between those MS patients that responded well and that lacked an *apoE4* allele was even more striking (see **Table 5, Exhibit D**).

13. I analyzed 51 patients suffering from stroke to determine if there is a relationship between *apoE* genotype and prognostic outcome. The study group was composed of Caucasian females currently diagnosed as suffering from stroke and currently under treatment with either aspirin or anti-thrombotic drugs (e.g. ticlopidine (TICLID™)). The patients in this study had an *apoE* allele distribution similar to a randomized North American-population (see **Table 6, Exhibit E**). To determine the stroke patient's *apoE* genotype, 5 mLs of whole blood were drawn from each stroke patient and used as a source of genetic material for *apoE* allele determination. The patient's *apoE* genotype was then compared to the speed of the patient's recovery from a stroke incident and the duration of their rehabilitation. I did not observe a negative outcome in stroke patients that carried an *apoE4* allele using these parameters.

14. My results and the subsequent results of others demonstrate that the Apolipoprotein E gene is linked to prognostic outcome in Alzheimer's disease, as well as numerous other neurological disorders, such as Parkinson's disease, multiple sclerosis, and stroke. Thus, I have shown that, in general, there are two genetically distinct types of patients with neurological disorders: those lacking the *apoE4* allele or ApoE4 protein isoform who are likely to have improved prognoses; and those having an *apoE4* allele or ApoE4 protein isoform who exhibit relatively poor prognoses.

15. My co-authors in Poirier *et al.*, "Apolipoprotein E4 allele as a predicator of cholinergic deficits and treatment outcome in Alzheimer disease," Proceedings of the National Academy of Sciences, U.S.A., 92:12260-12264, 1995, Marie-Claude Delisle, Remi Quirion, Isabelle Aubert, Martin Farlow, Debmoi Lahiri, Siu Hui, Philippe Bertrand, Josephine Nalbantoglu, Brian M. Gilfix, and Serge Gauthier, worked under my direction and control and did not contribute to the now claimed invention.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: APRIL 29TH 2003

Judes Poirier, Ph.D.

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Apolipoprotein E genotype related differences in brain lesions of multiple sclerosis

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Abstract

***Objectives*—Clinical reports have speculated on a more severe course of multiple sclerosis in patients with the apolipoprotein E (apoE) ε4 allele. As this could be reflected by differences in the severity of tissue damage MRI was used to obtain further support for a disease modifying effect of the apoE genotype.**

***Methods*—Brain MR scans of 83 patients (mean age 35.5 (SD 9.5 years) who participated in a cross sectional study on the distribution of genotype patterns in multiple sclerosis. The total lesion load on proton density weighted (T2-LL) and T1 weighted scans (T1-LL) obtained with conventional spin echo sequences at 1.5 T was measured. A "black hole" ratio ((T1-LL/T2-LL)×100) was also calculated. This indicates the proportion of multiple sclerosis lesions with more severe tissue damage and may reflect disease aggressiveness or quality of repair.**

***Results*—Patients with the apoE-ε3/ε4 genotype (n=19) showed a non-significantly greater T2-LL (16.0 (SD 14.0) cm^3) than patients with the ε2/ε3 (n=11; 13.3 (9.5) cm^3) or the ε3/ε3 genotype (n=49; 9.4 (SD 9.2) cm^3). Both the T1-LL (2.6 (SD 3.3) *v* 1.6 (SD 2.4) and 1.2 (SD 3.0) cm^3; p=0.04) and the black hole ratio (14.3 SD 11.9) *v* 7.4 (SD 9.3) and 8.4 (SD 13.3)%; p=0.02), however, were significantly higher in ε3/ε4 patients. Similar differences were seen when comparing patients with at least one ε4 allele with the remainder of the group.**

***Conclusions*—These data support speculations on a modulation of multiple sclerosis severity by the apoE genotype which can be attributed to more extensive tissue destruction or less efficient repair in carriers of the ε4 allele.**

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Keywords: multiple sclerosis; apolipoprotein E genotype; magnetic resonance imaging

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Multiple sclerosis is the most frequent neurological autoimmune disorder and the leading cause of serious neurological disability in early adulthood. Prevalence rates of multiple sclerosis vary substantially throughout the world and this has been attributed to environmental and genetic factors.[1–3] However, genetic factors may not only influence a person's susceptibility for multiple sclerosis but can also be expected to modulate the course of the disease.[4 5] In this context the apolipoprotein E genotype could play some part.

Apolipoprotein E (apoE) is a polymorphic plasma glycoprotein that transports cholesterol and other lipids.[6] The gene for apoE is located on chromosome 19 and is highly polymorphic. The three most common alleles are ε2, ε3, and ε4, which encode the three main isoforms of apoE: E2, E3, and E4. In various cell lines apoE3 has been shown to increase the growth and branching of neurites, whereas apoE4 was found to have the opposite effect.[7] At least equally important in the context of multiple sclerosis, there have been several reports indicating that apoE is involved in lipid redistribution after demyelination.[8] Therefore, an isoform specific differential effect of apoE may not only affect the preservation of axons but also the process of remyelination. Accordingly, first reports based on clinical assessment of severity of disease showed some trend towards a more aggressive course of multiple sclerosis in apoE ε4 carriers.[9 10] This prompted us to use brain MRI for further study of this hypothesis.

As known from treatment trials in multiple sclerosis MRI can provide objective markers of the burden of disease such as the total lesion load which may be even more sensitive for determining the extent of brain damage than clinical measures.[11] Furthermore, comparative analysis of lesion load as shown on various MRI sequences could serve to detect differences in the severity of tissue destruction between genotypes. Whereas multiple sclerosis lesions appear almost uniformly hyperintense on proton density and T2 weighted MRI only those lesions with more severe tissue loss are seen on T1 weighted scans.[12] These lesions have been termed "black holes" and the grade of MRI lesion hypointensity was shown to correspond well with the magnitude of axonal damage, extracellular oedema, and the degree of demyelination or remyelination.[13 14]

Methods

Over a period of 5 months we obtained blood samples for apoE genotyping from all patients admitted to our multiple sclerosis outpatient clinic who met Poser's criteria of laboratory supported or clinically definite multiple sclerosis.[15] Eighty nine of these 149 patients had undergone MRI at our institution. Six patients had to be excluded because of incomplete data or limited quality of the examinations. This left 83 patients (47 women, 36 men) with a mean (SD) age of 35.7 (9.5) years (range 17—60 years). Seventy six patients had

Table 1 Demographic and clinical findings in apoE genotype subgroups

	All (n=83)	ε2/ε3 (n=11)	ε3/ε3 (n=49)	ε3/ε4 (n=19)
Age (y)	35.7 (9.5) [34]	38.7 (3.0) [39]	35.9 (8.6) [35]	34.0 (9.6) [34]
Women (%)	58	55	59	58
MS duration (months)	90.5 (80.1) [77]	103.0 (142.6) [77]	91.0 (71.3) [77]	88.8 (62.0) [82]
EDSS score	1.9 (1.6) [1.5]	2.0 (1.7) [2]	1.8 (1.4) [1.5]	2.4 (2.2) [1.5]
Relapses (number)	6.2 (4.6) [5.0]	4.8 (3.6) [4.0]	6.2 (3.9) [6.0]	7.3 (6.6) [5.5]
RR course (%)	90	100	96	79

Data are given as mean (SD) [median].
EDSS=Expanded disability status scale; RR=relapsing-remitting; MS=multiple sclerosis.

a relapsing-remitting course of multiple sclerosis, five were in the secondary progressive phase and two patients had primary progressive multiple sclerosis. After informed consent, all patients were examined by one neurologist (SS-F) who recorded the pertinent demographic data, duration, and course of the disease,[5] disease severity according to the Kurtzke expanded disability status scale,[16] and the number of relapses. Relapses were defined as new or worsening neurological signs or symptoms with a duration of more than 24 hours. Retrospective data were confirmed by a review of the patient's charts.

All MRI examinations were performed on a 1.5 Tesla magnet (Philips Medical Systems, Eindhoven, The Netherlands) with a standard protocol using conventional spin echo sequences. This includes proton density and T2 weighted images (repetition time (TR))/echo time (TE): 2500 ms/30 and 90 ms) and T1 weighted images (TR/TE 600 ms/15 ms) in the axial plane. The T1 weighted sequence was performed after injection of gadolinium-DTPA in a dosage of 0.1mmol/kg bodyweight. The slice thickness was 5 mm. The mean interval between MRI and the clinical examination at the time of blood sampling was 4.4 months.

Morphological analysis was performed on the PD weighted and T1 weighted images. Firstly, an experienced observer (FF) marked all lesions on hard copies. To improve reproducibility and concentrate on more marked tissue changes we used a stringent definition for black holes—that is, lesion hypointensity had to be at least between that of grey matter and CSF or lower. Areas of mild hypointensity were not considered in the T1 analysis. Lesion measurement was then performed by another investigator (CE) using "DispImage", an image processing software provided by David Plummer, University College, London, UK, which has been described in detail elsewhere.[17 18] Using this technique, the intraobserver variability was 1.2% for measurements of the total volume of hyperintensities—that is, the T2 lesion load (T2-LL) and 4% for the entire "black hole" volume or T1 lesion load (T1-LL) in this study. This corresponds well with the intra-observer agreement reported by others.[17] We also calculated a "black hole" ratio (BHR; (T1-LL/T2-LL) x 100) to indicate the proportion of more severe tissue destruction among multiple sclerosis lesions. All these analyses were performed without knowledge of the patients' clinical condition or genotype.

ApoE genotyping was done according to the method of Hixon and Vernier[19] after extraction of high molecular weight DNA from peripheral blood.

STATISTICAL ANALYSIS

We used the statistical package for social sciences (SPSS/PC+) for data analysis. Categorical variables among the different apoE genotypes were compared by χ^2 tests. We used Levene's test to confirm a normal distribution of continuous variables. Comparisons of continuous variables between genotypes were conducted by Student's t test or with the non-parametric Mann-Whitney U test in the absence of a normal distribution.

Results

The apoE genotype frequencies were as follows: ε2/ε2 in two (2.4%), ε2/ε3 in 11 (13.3%), ε3/ε3 in 49 (59%), ε2/ε4 in one (1.2%), ε3/ε4 in 19 (22.9%), and ε4/ε4 in one (1.2%). This distribution of genotypes is in Hardy-Weinberg equilibrium. Consequently, the frequencies for the ε2, ε3, and ε4 allele were 16, 128, and 22.

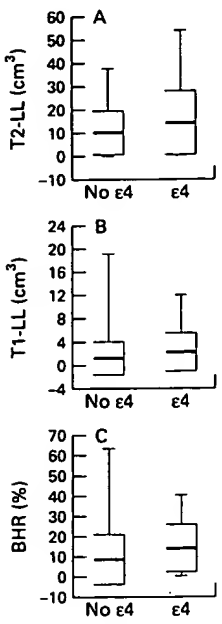
Demographic and clinical data for the total cohort and the ε2/ε3, ε3/ε3, and ε3/ε4 subsets at the time of blood sampling are shown in table 1. Patients with the ε3/ε4 genotype tended to be younger and had the highest mean EDSS score and number of relapses despite the shortest disease duration. A relapsing-remitting course was less frequent at examination in the presence of an ε3/ε4 genotype. However, none of these clinical differences reached significance.

The clinical data at the time of MRI showed a distribution of genotypes which was similar to that seen at the time of blood sampling. Results of the MRI analysis are shown in table 2.

Table 2 MRI findings in apoE genotype subgroups

	All (n=83)	ε2/ε3 (n=11)	ε3/ε3 (n=49)	ε3/ε4 (n=19)
T2 lesion load (cm³)	11.6 (10.7) [8.7]	13.3 (9.5) [14.4]	9.4 (9.2) [5.6]	16.0 (14.0) [11.3]
T1 lesion load (cm³)	1.6 (3.0) [0.4]	1.6 (2.4) [0.6]	1.2 (3.0) [0.1]	2.6 (3.3) [1.1]*
"Black hole" ratio (%)	9.6 (12.5) [5.8]	7.4 (9.3) [6.2]	8.4 (13.3) [4.0]	14.3 (11.9) [12.4]*
Active scans (number)	18	3	13	1†

Data are given as mean (SD)[median].
*ε3/ε4 *v* ε3/ε3, $p<0.02$ (Mann-Whitney U test).
†ε3/ε4 *v* ε3/ε3, $p<0.05$ (χ^2 test).



Box plots of MRI measures of multiple sclerosis lesion load in patients with and without at least one apoE ε4 allele.

Patients with the ε3/ε4 genotype had the greatest T2-LL. Differences in the T1-LL were even more pronounced and statistically significant between the ε3/ε4 and the ε3/ε3 genotype subgroups (p=0.018). Moreover, the black hole ratio was almost twice as high in patients with the ε3/ε4 genotype than in the other genotypes. Again this difference reached significance between ε3/ε4 and ε3/ε3 subsets (p=0.014). Interestingly, active scans as indicated by the presence of contrast enhancing lesions were noted less often in ε3/ε4 patients, however (p=0.048). Comparison between carriers of at least one ε4 allele to the remainder of the study population confirmed a trend towards a higher T2-LL (15.1 (SD 13.7) *v* 10.4 (SD 9.2) cm^3, p=0.24) and a significantly greater T1-LL (2.4 (SD 3.2) *v* 1.3 (SD 2.8) cm^3, p=0.044) and black hole ratio (13.3 (SD 11.8) *v* 8.3 (SD 12.5)%, p=0.024) in ε4 patients (figure A–C).

Discussion

Earlier studies on the apoE genotype in multiple sclerosis have dealt primarily with allele frequencies and found no significant differences in the distribution of genotypes between patients with multiple sclerosis and the normal population.[9 20 21] However, a more aggressive course of multiple sclerosis in patients with the apoE ε4 allele has been suggested by different investigators.[9 10] This was not confirmed in a recent study on 361 patients with clinically definite multiple sclerosis.[22] Morphological findings of the present study add to this controversy.

Using MRI in a cross sectional study design we found the highest lesion load in patients with multiple sclerosis with the apoE ε3/ε4 genotype. This difference was more pronounced for the T1-LL than the T2-LL and was significant between the ε3/ε4 and the ε3/ε3 subgroups. In parallel there was also a significant difference between these genotypes for the relative proportion of "black holes". The black hole ratio of patients with the ε3/ε4 genotype was almost twice that of patients with an ε2/ε3 or ε3/ε3 genotype. Similar and significant differences were noted when comparing all patients with at least one ε4 allele to the remainder of the study population. Histopathological studies have shown that black holes represent multiple sclerosis lesions with more severe tissue destruction.[13 14] Furthermore, using MR spectroscopy a direct correlation was found between the extent of hypointensity of multiple sclerosis lesions on T1 weighted images and the relative reduction in N-acetylaspartate, which is considered a marker for axonal density.[23] Therefore, our findings of a higher T1-LL and higher black hole ratio can be viewed as indirect evidence for a less favourable course of multiple sclerosis in patients carrying the ε4 allele. Clinically our patients with an apoE ε3/ε4 genotype also tended to have more aggressive disease with greater disability and a higher number of relapses experienced in a shorter period of time. These differences did not reach significance but are in line with the recent finding of a faster increase of disability in apoE ε4 carriers during 2 years of treatment with glatiramer acetate.[24]

Whether a higher proportion of T1-LL in apoE ε4 patients is due to more aggressive disease with extensive demyelination or axonal loss within acute plaques or a consequence of less efficient repair thereafter cannot yet be determined. More active multiple sclerosis in apoE ε4 carriers could be another explanation for our findings. This would be supported by their overall larger T2-LL and a somewhat higher number of relapses. However, a significantly lower frequency of active scans in patients with the apoE ε3/ε4 genotype argues against this assumption although this finding certainly suffers from the cross sectional design of our study and the relatively few patients examined. For these reasons and because the proportion of patients with any treatment was similar between genotype subgroups we also did not attempt to consider the possible influence of different treatments at the time of the MRI examination.

These preliminary results provide morphological support for a negative effect of the apoE ε4 allele on the course of multiple sclerosis. They also illustrate the capability of MRI to detect small differences between patient groups which clinically would have gone undetected. Although these data cannot serve to explain the reasons for divergent findings in previous clinical studies they indicate the need for further investigation of the the role of the apoE genotype in multiple sclerosis. Such efforts may also take advantage of the sensitivity and objective nature of MRI findings in the future.

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Apolipoprotein E Genotype and Outcome in Aneurysmal Subarachnoid Hemorrhage

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George K.C. Wong, MB, ChB; H.K. Ng, MD

Background and Purpose—Active management of ruptured intracranial aneurysm in subarachnoid hemorrhage is indicated in patients with favorable prognosis. Outcome prediction is based on patient characteristics and clinical and radiological factors. Current clinical grading scales are imprecise, with low interobserver reproducibility. Therefore, outcome prediction remains inconsistent and decision making becomes difficult, especially for patients with poor clinical grade.

Methods—The possible relationship between apolipoprotein E genotype and the outcome of patients suffering spontaneous subarachnoid hemorrhage was investigated. A prospective study was conducted on all patients with spontaneous aneurysmal subarachnoid hemorrhage admitted to our unit during a 2-year period. All patients were managed according to standard protocol, and treatments were given according to their clinical grading. Patient characteristics, clinical grade, radiological grade, and apolipoprotein E genotype were documented. The focus of the study was the 6-month neurological outcome for this group of patients after they were discharged.

Results—Seventy-two patients with aneurysmal subarachnoid hemorrhage were admitted to the Prince of Wales Hospital in Shatin, Hong Kong, China, from February 1998 to February 2000. Their ages ranged from 24 to 95 years of age, with a mean (SD) age of 58.3 (15.0) years. Apolipoprotein E ε4 was found in 15 patients (21%). At 6 months, Glasgow Outcome Scale score ≤3 was found in 29 patients (40%). Univariate analysis showed that older patients (odds ratio [OR], 1.03; 95% CI, 1.00 to 1.07; $P=0.07$) and patients with poor Fisher's grade (OR, 4.5; 95% CI, 1.3 to 15.2; $P=0.01$), poor World Federation of Neurological Surgeons grade (OR, 5.8; 95% CI, 1.9 to 17.8; $P=0.002$), or apolipoprotein E ε4 (OR, 6.0; 95% CI, 1.7 to 21.3; $P=0.006$) were more likely to attain unfavorable outcome at 6 months. The additional effect of apolipoprotein E ε4 remained significant in the multiple logistic regression model (OR, 11.3; 95% CI, 2.2 to 57.0; $P=0.003$); the gain in predictive performance was not significant ($P=0.26$).

Conclusions—Apolipoprotein E ε4 genotype is related to poor outcome in patients with subarachnoid hemorrhage. (***Stroke.*** **2002;33:548-552.)**

Key Words: apolipoproteins ■ outcome ■ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) accounts for 25% of all cerebrovascular deaths. The case fatality rate of SAH is reported to be as high as 50%.[1] Among the remaining survivors, 50% are left severely disabled. The etiology of 80% of the cases is ruptured intracranial aneurysm. Morbidity and mortality are largely due to rebleeding aneurysm and vasospasm.[2] Thus, early aneurysm intervention will facilitate both treatment of vasospasm and prevention of rebleeding. Currently, prediction of outcome relies on demographic, clinical, and radiological factors. However, accurate outcome prediction in aneurysmal SAH remains imprecise despite use of currently established clinical grading scales.[3,4]

Evidence from animal studies on apolipoprotein E genotype has suggested its important role in the response to nervous system injury.[5,6] The 3 most common genetic alleles in humans are apolipoprotein E ε2, ε3, and ε4 (APOE2, APOE3, and APOE4, respectively), which encode 3 isoforms of the apolipoprotein E2, E3, and E4, respectively. Several prospective clinical studies on the APOE4 allele have shown its association with poor outcome in patients with intracerebral hemorrhage and head injury.[7,8]

In a recent prospective case-control study by Kokubo et al, an association was suggested between APOE4 and SAH.[9] However, the sample size of patients with SAH in that study was small (n=37), and the association between apolipoprotein E genotype and outcome was not investigated. Our study was designed to test the hypothesis that APOE4 genotype is associated with unfavorable outcome in SAH patients. This

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may supplement other established outcome predictors with a more accurate outcome prediction.

Subjects and Methods

Patient Recruitment

A prospective clinical study was performed on all patients admitted to the Prince of Wales Hospital in Shatin, Hong Kong, China, with spontaneous aneurysmal SAH during a 2-year period from February 1998 to February 2000. The study was approved by the Ethics Committee of the Chinese University of Hong Kong and conducted in accordance with the Helsinki Declaration. CT or lumbar puncture was used to confirm the diagnosis of SAH. All SAH patients directly admitted or transferred from another hospital were clinically assessed by the neurosurgical team. The patients' characteristics, World Federation of Neurological Surgeons (WFNS) clinical grading score, and Fisher's grading score (quantifying the severity of SAH on CT) were recorded.[10,11] Blood samples for apolipoprotein E genotyping were obtained via venipuncture for other routine blood tests. Informed consent from patients or their next of kin was obtained before blood samples were taken.

Neurological Assessment

Assessment and classification of SAH patients were based on the grading scale as defined by the WFNS, as follows: grade 1, Glasgow Coma Scale (GCS) score 15; grade 2, GCS score 13 to 14 with no focal neurological deficit; grade 3, GCS score 13 to 14 with focal neurological deficit; grade 4, GCS score 7 to 12; and grade 5, GCS score 3 to 6. Patients with WFNS grade 1 to 3 were classified as patients with good WFNS grade (with favorable prognosis), and patients with grade 4 to 5 were defined as patients with poor WFNS grade (with unfavorable prognosis).

Management Protocol

All patients were managed under a standard protocol in either the neurosurgical high dependency unit or the intensive care unit if intubation and mechanical ventilation were required. Intravenous nimodipine 1 to 2 mg/h (Nimotop, Bayer) and the prophylactic anticonvulsant sodium valproate 400 mg/8 h (Epilim Intravenous, Sanofi Winthrop Industries) were started on admission. Systolic blood pressure was maintained at <140 mm Hg unless there was a known history of hypertension or the patient developed clinical vasospasm, in which cases higher systolic blood pressure ranges were accepted.

All patients admitted with spontaneous SAH were investigated within 24 hours, initially with the use of CT angiography to screen for intracranial aneurysm. Patients with a good WFNS grade (WFNS grade 1 to 3) would proceed to digital subtraction angiography if CT angiography was negative. If the patient initially presented with a poor WFNS grade (grade 4 or 5) or had negative CT angiography and subsequently improved to good WFNS grade, digital subtraction angiography would then be performed. Patients with persistent poor WFNS grade and negative CT angiography were not included in this study.

Treatment of Aneurysm

The treatment options were either endovascular aneurysm occlusion with Guglielmi detachable coils (GDC, Boston Scientific Corp) or microsurgical clipping of aneurysm. The decision of whether to use endovascular or surgical treatment depended on the configuration of the aneurysm (site, size, and shape), the patient's medical condition, and the family's or patient's preference. All definitive treatments were performed within 48 hours.

Determination of Apolipoprotein E Genotype

The salting out method was used to extract DNA from the venous blood sample. The apolipoprotein E genotype was determined by a polymerase chain reaction on an MJ Research PTC-200 thermal cycler followed by enzyme restriction and polyacrylamide gel electrophoresis, as described previously by 1 of the authors.[12]

Follow-Up and Outcome Assessment

Outcome assessment was based on the Glasgow Outcome Scale (GOS), as follows: grade 5, good recovery; grade 4, moderate disability: disabled but independent; grade 3, severe disability: conscious but disabled; grade 2, persistent vegetative state; and grade 1, death. Grades 4 and 5 were classified as favorable outcome, and grades 1 to 3 were classified as unfavorable outcome.[13]

A follow-up interview was arranged for 6 months after the patients were discharged to determine the clinical outcome according to the GOS. For those patients who were unable to attend the interview because of severe disability, consultation with their family or caregiver was made over the telephone. Throughout the study, the investigators were blinded to the patients' apolipoprotein E genotype. The patients' outcome assessment and apolipoprotein E genotyping results were submitted independently for statistical analysis.

Statistical Analysis

Univariate analysis was performed to determine the effect of age, sex, Fisher's grade, WFNS grade, and APOE4 on the outcome at 6 months (defined by GOS score), after which 2 prognostic models were developed. The first model (model 1) comprised all of the aforementioned key factors except APOE4, which was included in the second model (model 2), ie, age, sex, Fisher's grade, and WFNS grade. The model was performed by a stepwise multiple logistic regression analysis[14] with the use of the Akaike information criterion[15] as variable selection criterion. The second model (model 2) added the effect of APOE4 to model 1. The predictive ability of each model was assessed by the area under the receiver operating characteristic (ROC) curve.[16] An area under the ROC curve value of 0.5 indicates no predictive power, whereas a value of 1.0 indicates excellence in prediction. The areas under the ROC curves for both models were then compared according to the method developed by Hanley and McNeil.[17] The bootstrapping technique was used as an internal validation to correct for possible bias due to overfitting.[18] All analyses were performed with the use of S-Plus 2000 statistical software (MathSoft, Inc).

Results

Patient Characteristics

Seventy-two patients with aneurysmal SAH, confirmed by CT angiography or digital subtraction angiography, were recruited for analysis. Their ages ranged from 24 to 95 years, with a mean (SD) age of 58.3 (15.0) years. There were 47 women (65%) and 25 men (35%). The patients' characteristics, WFNS grade, Fisher's grade, and apolipoprotein E genotype are summarized in Table 1.

Outcome Analysis

Of the 72 patients, 8 patients died during the same admission, 54 patients attended the 6-month interview, 6 patients were unable to attend the interview in person but were represented by their caregivers over the telephone, and 4 patients required management in a convalescence hospital. At 6 months, 61 patients (84.7%) remained alive, 43 patients (59.7%) had a GOS score >3, and 29 patients (40.3%) had a GOS score ≤ 3. Results from the univariate analysis suggested that older patients were more likely to attain an unfavorable outcome (62.2 [17.2] versus 55.7 [12.9] years; odds ratio [OR], 1.03; 95% CI, 1.00 to 1.07). Twenty-five of 50 patients (50%) with poor Fisher's grade had significantly higher unfavorable 6-month outcome compared with only 4 of 22 patients (18.2%) with Fisher's grade 1 and 2 (OR, 4.5; 95% CI, 1.3 to 15.2). Similarly, more unfavorable outcomes were found in patients who presented with poor WFNS grades than those

TABLE 1. Clinical and Genetic Characteristics of Patients With Aneurysmal SAH

Characteristics	Patients (n=72)
Age, mean (SD), y	58.3±15.0
Sex, M/F	25/47
WFNS grade	
≤3 (Good grade)	52
≥4 (Poor grade)	20
Fisher's grade*	
1/2 (Good grade)	22
3/4 (Poor grade)	50
Apolipoprotein E genotype	
ε2/ε2	1
ε2/ε3	3
ε3/ε3	53
ε2/ε4	2
ε3/ε4	13
ε4/ε4	0

Values are number of patients unless indicated otherwise.

*The Fisher's grading system indicates the amount of subarachnoid blood on CT, with higher grades indicating larger amount of subarachnoid blood and thus a higher risk of developing vasospasm.

with good WFNS grades (70% versus 28%; OR, 5.8; 95% CI, 1.9 to 17.8) and for those with APOE4 compared with those without the allele (73.3% versus 31.6%; OR, 6.0; 95% CI, 1.7 to 21.3) (Table 2).

The multivariable relationships between the variables, without (model 1) and with (model 2) the effect of APOE4, and outcome at 6 months are shown in Table 3. The results indicate that APOE4 (P=0.003; OR, 11.3; 95% CI, 2.2 to 57.0) remained significant in predicting outcome, after adjustment for other known risk factors. Model 2 had a

TABLE 2. Distribution of Characteristics at 6-Month Outcome in Patients With SAH

	6-Month Outcome*		Univariate Analysis	
Variable	Unfavorable (n=29)	Favorable (n=43)	OR (95% CI)	P
Age, y	62.2±17.2	55.7±12.9	1.03 (1.00–1.07)	0.07
Sex, M/F	12/17	13/30	1.6 (0.6–4.4)	0.33
WFNS grade				
≤3†	15	37	...	
≥4	14	6	5.8 (1.9–17.8)	0.002
Fisher's grade				
1/2†	4	18	...	
3/4	25	25	4.5 (1.3–15.2)	0.015
APOE4 heterozygous				
No†	18	39	...	
Yes	11	4	6.0 (1.7–21.3)	0.006

*Six-month outcome defined by GOS score: GOS 1, 2, and 3=unfavorable; GOS 4 and 5=favorable.

†Reference group.

TABLE 3. Multiple Logistic Regression, Without and With APOE4, in Predicting Unfavorable Outcome (GOS ≤3) in Patients With SAH

	Model 1		Model 2	
Variable	OR (95% CI)	P	OR (95% CI)	P
Age	1.04 (1.00–1.08)	0.04	1.04 (1.00–1.08)	0.058
WFNS grade				
≤3*	...		...	
≥4	5.8 (1.6–20.6)	0.006	7.6 (1.9–30.3)	0.004
Fisher's grade				
1/2*	...		...	
3/4	3.3 (0.9–12.5)	0.078	5.2 (1.0–25.6)	0.042
APOE4 heterozygous				
No*			...	
Yes			11.3 (2.2–57.0)	0.003

	Model 1	Model 2	P
Area under the ROC curve (bootstrap corrected)†	0.78	0.83	0.26‡

*Reference group.

†From 1000 bootstrap samples.

‡Comparison of area under the ROC curve between model 1 and model 2.

bootstrap-corrected area under the ROC curve greater than model 1 (83% versus 78%). The addition of APOE4 to the prognostic model improved the predictive performance by 5%. However, such an improvement was not statistically significant (P=0.26).

Discussion

The results of this study demonstrate a clear relationship between APOE4 and poor outcome in aneurysmal SAH. This is the first prospective clinical study that had shown such a relationship. In a similar study by Dunn et al,[19] such an effect of APOE4 on the 6-month outcome of those SAH patients admitted to the regional neurosurgical unit was not shown. The authors stated that the negative findings were due to selection bias, and no definite conclusion regarding the effect of APOE4 on SAH patients could be drawn. Evidence from previous experimental and clinical studies on apolipoprotein E genotype supports this interpretation. Apolipoprotein E is a known "injury factor" in the central nervous system, and previous proposed mechanisms include isoform-specific immunomodulatory,[20] neurotoxic,[6] and oxidative effects.[21]

In the experimental study performed on microglial cells by Barger and Harmon,[20] apolipoprotein E4 was shown to be less effective in suppressing microglia-mediated neurotoxicity than apolipoprotein E3. In the transgenic work of Buttini et al,[6] apolipoprotein E3 was shown to be a neuroprotective factor, but, in contrast, apolipoprotein E4 was shown to inhibit this beneficial function of apolipoprotein E3. Prospective clinical studies by Teasdale et al[8] and Alberts et al[7] suggest that APOE4 is associated with higher mortality and morbidity in head injury and nonaneurysmal intracerebral hemorrhage, respectively. Their findings indicate that apolipoprotein E4 exerts an inhibitory effect on neural recovery after brain injury. These studies support our findings that the

patients in our study who possess the APOE4 allele are more susceptible to an unfavorable outcome. The exact mechanism of apolipoprotein E4 still remains unknown.

The brain damage of SAH results from both the initial hemorrhage and the subsequent ischemia secondary to vasospasm. Vasospasm is the main cause of secondary brain injury in SAH. The results of our study indicate that apolipoprotein E4 may act directly on the effect of brain ischemia, which accounts for the poorer outcome in SAH patients. However, several previous studies have shown no evidence of association between APOE4 and outcome in patients with ischemic stroke.[22,23] To explain the adverse prognostic effect of APOE4 on SAH patients, apolipoprotein E4 must act either indirectly or through a mechanism other than ischemia.

One study on animals has shown that the products of hemolysis in the subarachnoid space after SAH may lead to widespread necrosis of the cortex.[24] If apolipoprotein E4 exerts its effect on the mechanism via products of hemolysis rather than directly on ischemic brain, this may provide an explanation for the divergent effects of APOE4 on the outcome of patients with hemorrhagic and ischemic stroke. This may also explain the adverse effect of APOE4 on patients with intracerebral hemorrhage, head injury, and SAH because all these patients may have blood in either the subarachnoid space (spontaneous and traumatic SAH) or within the brain parenchyma (hematoma and contusions), whereas the effect of APOE4 may be absent in patients with ischemic stroke as long as hemorrhagic transformation does not occur. The study by Motto et al[25] addressed the importance of hemorrhagic transformation on the poorer outcome of patients with ischemic stroke. Their findings may support our interpretation with the caveat that the exact mechanism of APOE4 remains to be defined.

On the other hand, apolipoprotein E4 may have an indirect effect on causing vasospasm in SAH. Endothelin-1 is known to be one of the most potent vasoconstrictors in SAH. In the study by Fassbender et al,[26] endothelin-1 was found to be released from cerebrospinal fluid leukocytes during the acute phase of SAH. The animal study by Paris et al[27] suggested that there is a synergistic relation between apolipoprotein E and endothelin-1 in vasoconstriction. This synergistic effect was especially strong with apolipoprotein E4 compared with apolipoproteins E2 and E3. Therefore, the effect of apolipoprotein E4 on SAH patients may be due to its synergistic effect with endothelin-1 during the acute phase of hemorrhage in causing widespread and persistent vasospasm. This may also explain the insignificance of APOE4 in ischemic stroke since vasospasm does not form an important part of the pathogenesis of secondary brain damage in this situation.

The frequency of APOE4 allele varies across different ethnic groups, with the frequency of APOE4 allele in the Chinese population reported to be between 5% and 8%.[12,28,29] This is much lower than the average white population figure of approximately 20%.[30] In our study, after exclusion of 1 white patient, the frequency of 1 APOE4 allele was 21.1%. If we assume that the previously reported frequencies of APOE4 in the Chinese population are accurate, this implies a possible link between APOE4 allele and SAH. Similar findings have been found between APOE4 and SAH patients in the Japanese population.[9]

Alternatively, the finding of comparatively high APOE4 allele frequency in SAH patients may be due to age bias in previous reports on the Chinese population, which mainly focused on elderly patients. There is evidence to suggest that the frequency of APOE4 allele may be less in the elderly group.[31]

There were several drawbacks in our study. The frequency of APOE2 homozygote was reported to be approximately 0.8% in the white population.[30] In our study the sample size was too small to gather enough patients with the APOE2 genotype. Evidence has shown that APOE2 has a neuroprotective effect, but in our study only 1 patient harbored 2 APOE2 alleles, and it was therefore difficult to draw any conclusions regarding its impact on outcome.[32,33] In addition, none of the patients in our study were APOE4 homozygous, making it impossible to assess the gene dose effect on outcome prediction. The areas under the ROC curves of our prognostic models (78% excluding APOE4 and 83% including the genotype) indicated a good predictive performance in poor outcome prediction, although the addition of APOE4 effect did not show any statistically significant improvement in the prognostic model. This may be due to the small sample size of our study, which may also explain the wide CIs obtained in our results. Thus, to yield a better estimation and prediction of 6-month neurological outcome, a larger or possibly a multicenter study would need to be performed.

The results from this study offer a new perspective on outcome prediction for patients with SAH. With currently available technologies in DNA extraction and amplification, apolipoprotein E genotyping can be achieved within 20 hours. Determination of the apolipoprotein E genotype, in combination with other clinical data, might be clinically useful in determining the patient's prognosis and facilitating subsequent decision making on the definitive management strategy in SAH patients. A multicenter prospective study with a larger sample size is necessary to investigate the gene dose effect of APOE4 and the neuroprotective effect of APOE2 in SAH patients. A large sample size will also enable us to further investigate the contributory effect of APOE4 to the outcome predictive model. Further studies on the Chinese population and other ethnic groups are required to confirm our findings of a higher frequency of APOE4 in patients with SAH.

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Association of *APOE* ε4 allele with survival in amyotrophic lateral sclerosis

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Abstract

APOE ε4 allele is associated with poorer outcome in degenerative neurological diseases. Its role in amyotrophic lateral sclerosis (ALS) is still unclear. The aim of the present study was to further analyze the association of *APOE* ε4 allele with progression and survival of ALS.

One hundred consecutive ALS patients (53 males) and 133 controls were genotyped for the *APOE* ε4 allele. The association of this allele with survival to death or tracheostomy was analyzed by Kaplan–Meier survival analysis.

The frequency of the *APOE* ε4 allele in ALS patients was slightly higher (15.1%) than in the control group (10.9%). Patients with or without an *APOE* ε4 allele had a similar age of onset and frequency of bulbar onset. There was a significant shortening of the 50% probability of survival (by 32 months) in patients carrying the *APOE* ε4 allele ($p = 0.03$).

In conclusion, carrying an *APOE* ε4 allele is a poor prognostic factor in ALS. This is compatible with a role of apolipoprotein on neuronal survival and repair. © 2001 Published by Elsevier Science B.V.

Keywords: Amyotrophic lateral sclerosis; Motor neuron disease; Apolipoprotein E ε4 allele

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative motor neuron disorder affecting both the upper and the lower motor neurons. The pathogenesis of sporadic ALS, the most common form of disease, is unknown and probably multifactorial, including overexposure of motor neurons to glutamate and oxidative stress-induced cell death. Further causative factors, including a genetic predisposition, may have a modulatory influence [1].

The rate of progression of the disease is fast, but can differ strikingly among affected individuals, ranging from few months to 10 years or more. Older age and a bulbar form at disease onset are usually associated with more rapid deterioration.

Apolipoprotein E (ApoE) is a key regulator of plasma lipid levels, affecting all lipoproteins by modulating their clearance or their processing, as well as the production of hepatic very low density lipoproteins [2]. The human *APOE* gene has three common isoforms: ε2, ε3 and ε4. ApoE is produced in abundance in the brain, and it is the principal lipid transport protein there [3]. Roses [4] found that the *APOE* ε4 allele is a major susceptibility gene for Alzheimer's disease (AD), associated with about 50% of cases of sporadic and familial AD.

Because of the apparent similarities between AD and ALS regarding the pathogenesis of these diseases, several authors have studied the association of *APOE* ε4 and ALS, and reached conflicting results. Some studies [5–9] found the frequency of the *APOE* ε4 allele to be comparable to the allele frequency of the general population and did not find any association between the age at onset, site of onset, or duration of the disease in carriers or non-carriers of an *APOE* ε4 allele. In other studies, an *APOE* ε4 allele was associated with poor clinical outcome in ALS patients. One study showed a significantly shorter duration of disease for patients carrying the ε4 allele [10]; in another study, a significantly more rapid progression rate was seen in the initial stage of disease for patients with the ε4 allele [6]. Two other studies showed a trend toward

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Table 1
Demographic data of ALS patients with and without *APOE* ε4 alleles

	APOE non-ε4	*APOE* ε4	Total
Number of patients	71	29[a]	100
Male, %	36 (51%)	17 (59%)	53 (53%)
Ashkenazi, %	43 (61%)	18 (62%)	61 (61%)
Age of onset (years, mean ± S.E.)	57 ± 13	58 ± 30	58 ± 13
Bulbar onset, %	15 (21%)	9 (31%)	24 (24%)

[a] Including two homozygous patients.

shorter survival or more rapid disease progression for patients with the ε4 allele, but without statistical significance [7,11]. A higher proportion of bulbar onset patients was also seen in *APOE* ε4 carriers [10,11]. In the present study, we examined the *APOE* ε4 allele frequency in a large cohort of ALS patients and correlated its presence to survival.

2. Patients and methods

The study included 100 consecutive sporadic ALS patients in Israel (53 males). Their mean age was 57 ± 12 years, range 21 to 82 years. Of the 100 patients, 61 were Ashkenazi, 34 were non-Ashkenazi Jews and 5 were non-Jews. Twenty four patients had a bulbar form at onset; the remaining 76 had a limb onset. All patients were diagnosed as definite or probable ALS using the revised El Escorial criteria [12]. The frequency of the *APOE* ε4 allele in the ALS population was compared to a previously published group of healthy volunteers of similar ethnicity [13]. This group constituted of 133 subjects (58 males) aged 70 ± 12 years, without any known neurological disease.

DNA was extracted from patients' lymphocytes. *APOE* genotype was determined by polymerase chain reaction (PCR), followed by Afl III digestion and electrophoresis, as previously described [14].

Survival was defined as time to death or tracheostomy. Analysis of survival was based on Kaplan–Meier curves using SPSS-PC software and the log rank test used to compare groups.

3. Results

Table 1 shows the demographic data of patients with and without *APOE* ε4 alleles. There were 29 patients who had at least one *APOE* ε4 allele (2 were homozygotes). The frequency of *APOE* ε4 alleles in the ALS group was 15.1%, as opposed to only 10.9% in the control group [13]; however, this difference was not statistically significant ($p = 0.13$, χ^2 test). Patients with and without *APOE* ε4 alleles had similar male/female and Ashkenazi/non-Ashkenazi ratios. The age at onset in the two patient groups was similar (Table 1). The percent of patients with a bulbar form at onset was higher in *APOE* ε4 (9/29) than in non-*APOE* ε4 carriers (15/71); however, this difference was not statistically significant ($p = 0.27$, χ^2 test).

There was no difference in the median duration of follow up (to death/tracheostomy or to the end of the study) between patients with and without *APOE* ε4 alleles (38 vs. 36 months, respectively). By using Kaplan–Meier survival analysis, we found that the survival of patients carrying at least one *APOE* ε4 allele was significantly shorter compared to patients not carrying this allele. The time to reach a 50% probability of survival for patients carrying at least one *APOE* ε4 allele was 39 months (95% confidence interval 28.5–49.5), while in patients with no *APOE* ε4 allele, this landmark was reached after 71 months (95% confidence interval 24.6–117.4) (Fig. 1, $p = 0.03$, one-tail log rank test). This difference was similar for limb onset patients.

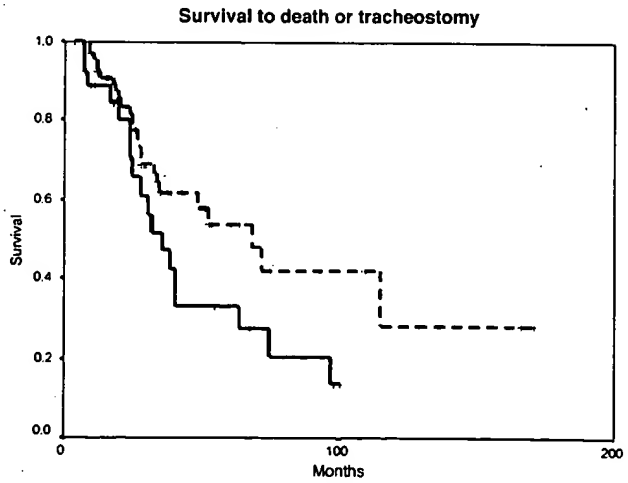

Fig. 1. Kaplan–Meier curves of survival for ALS patients with and without *APOE* ε4 alleles. Solid line: *APOE* ε4, dash line: *APOE* non-ε4. Patients carrying at least one *APOE* ε4 allele ($n = 29$) reached 50% probability of survival 32 months before patients not carrying this allele ($n = 71$, $p = 0.03$).

4. Discussion

The main finding in the present study was that the *APOE* ε4 allele is associated with poor survival in ALS. There is a proven association between *APOE* ε4 allele and another neurodegenerative disease—AD [4,13]. The *APOE* ε4 allele was also associated with poor clinical outcome after head injury [15], in dementia associated with stroke [16,17] and with earlier age at onset of Wilson's disease [18]. However, in Parkinson's disease, there is no obvious relationship between clinical factors of progression and occurrence of dementia and *APOE* ε4 [19,20], although previous reports found an earlier age of onset in patients carrying *APOE* ε4 alleles [21].

The association between *APOE* genotype and neurodegenerative disorders correlates with recent findings suggesting that ApoE has an important role in neuronal homeostasis and repair, and that ApoE ε3 and ε2 proteins may mediate neuronal repair processes, whereas ApoE ε4 may be less effective or even toxic [2].

The finding of a slightly higher frequency of *APOE* ε4 alleles in our ALS group is in agreement with other studies [5,11], which showed that the frequency of the *APOE* ε4 allele in ALS patients was slightly elevated, but not enough to reach statistical significance. Interestingly, Mui's group [5] included both sporadic and familial ALS cases. In familial cases, in which a specific genetic mutation causes the disease, the effect of *APOE* genotype is possibly less significant. In that series, the frequency of *APOE* ε4 alleles was increased more markedly in the sporadic ALS group, in comparison to the familial group. In order to evaluate a genetic modulating factor in a presumably multifactorial disease like ALS, it may be more appropriate to examine only sporadic ALS patients, as done by us.

It has been suggested that patients with a bulbar form at onset have a more rapidly progressive disease. Consequently, we compared frequencies of *APOE* ε4 alleles in patients with bulbar onset with those with limb onset and found a higher percentage of bulbar onset patients in the *APOE* ε4 group; however, this was not significant statistically, similarly to previously reported data [10]. It has to be mentioned, however, that in our patient population, patients with limb and bulbar onset had a similar survival, independently of their *APOE* status (data not shown).

In AD, the existence of an *APOE* ε4 allele was demonstrated to lower the age of onset of the disease [22]. In our ALS group, as reported also previously [5,7,10,11], there was no effect of *APOE* on age of onset; however, there was a significant negative effect on survival. This discrepancy of the effects of *APOE* genotype on AD and ALS may be due to the time of diagnosis in these diseases. In AD, in which the clinical diagnosis is thought to be preceded by a long period of biological deterioration, the *APOE* genotype may influence the age of onset. In contrast, in ALS, in which the preclinical period is probably much shorter, the effect of *APOE* genotype is not seen on the age of onset, but rather on survival. This is in agreement with other neurological diseases like multiple sclerosis [23], in which there is a relatively short preclinical phase and the effect of *APOE* genotype was found to be predominantly on the disease progression and less on the age of onset.

It is interesting to note that *APOE* ε4 has a different effect in ALS (causing more rapid progression) than in AD (advancing age of onset), since a similar discrepancy exists also among other genetic factors modulating these diseases: ALS patients with SOD mutations and sporadic ALS patients have a similar age of onset and disease duration [24], whereas AD mutations in amyloid precursor protein (APP) and presenilin genes are associated with an earlier disease onset in comparison to sporadic AD cases, yet they do not affect disease duration [22].

This study shows that ALS patients carrying an *APOE* ε4 allele have a significantly shorter survival. This finding is comparable with other central nervous system diseases as faster decline of cognitive performance in Down's syndrome [25] and faster progression of disability in multiple sclerosis subjects [23,26–28], and of diseases of the peripheral nervous system, as diabetic neuropathy and human immunodeficiency virus related neuropathy [29].

The biological effect of *APOE* ε4 in ALS and other neurodegenerative diseases may be explained by the recent findings that the ApoE protein has antioxidant activity, and the efficacy of ApoE ε4 protein is lower, compared to that of ApoE ε2 and ε3 proteins [30], as well as ApoE plays a role in detoxification of lipid peroxidation [31].

In conclusion, we demonstrated that the *APOE* ε4 genotype is a poor prognostic factor in ALS, as it is associated with a shorter survival. The mechanism by which it influences the disease course is not known and deserves further study.

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Exhibit A

Table 1. ApoE Genotype Distribution of Study Group, a Larger Population, and Across Different Age Groups

Genotype	% population (Canada)	% Study	40-50	60	70	80
E4	15.20%	16.3%	18.8%	10.7%	20.0%	0.0%
E3	77%	68.8%	68.8%	71.4%	70.0%	100.0%
E2	7.80%	15.0%	12.5%	17.9%	10.0%	0.0%

Exhibit B

Table 2. Drug Response of Parkinson's Disease Patients with Different apoE Genotypes

Genotype	% population (Canada)	% Study	Bad responder		Good responder
E4/E4	3.9%	0%	0%		0%
E4/E3	20.6%	17%	20%	←	8%
E4/E2	9.8%	5%	4%		4%
E3/E3	61.8%	63%	68%	→	72%
E3/E2	2.0%	14%	4%	→	16%
E2/E2	2.0%	2%	4%		0%

Genotype	% population (Canada)	% Study	Bad responder		Good responder
+E4	15.2%	16%	18%	←	9%
-E4	84.8%	84%	81%	→	91%

Exhibit C

Table 3. apoE Genotype Distribution of Study Group, Larger Population, and Amongst Different Age Groups

Genotype	% population (Canada)	% study	<30	30-40	>40
E4	15.20%	15.6%	9.7%	21.6%	12.5%
E3	77%	66.7%	67.7%	62.2%	68.8%
E2	7.80%	17.8%	22.6%	16.2%	18.8%

Exhibit D

Table 4. apoE Genotype Distribution in MS Patients Responding Well to Drug Therapy Versus Those Patients Responding Poorly

Genotype	% population (Canada)	% study	Bad responders		Good responders
E4/E4	3.9%	0.0%	0.0%		0.0%
E4/E3	20.6%	15.4%	8.3%		8.3%
E4/E2	9.8%	6.2%	6.9%	←	1.7%
E3/E3	61.8%	60.0%	23.3%	→	33.3%
E3/E2	2.0%	16.9%	5.0%	→	13.3%
E2/E2	2.0%	1.5%	1.7%		0.0%

Table 5. apoE Allele Load in MS Patients Responding Well to Drug Therapy Versus Those Patients Responding Poorly

Genotype	% population (Canada)	% study	Bad		Good
+E4	15.2%	16.5%	6.7%	←	5.6%
-E4	84.8%	65.9%	20.0%	→	43.3%

Exhibit E

Table 6. apoE Allele Distribution of Study Group Compared to Representative Population

Genotype	% Population (Canada)	Study Group
E4/E4	3.9%	0%
E4/E3	20.6%	27%
E4/E2	9.8%	2%
E3/E3	61.8%	59%
E3/E2	2.0%	12%
E2/E2	2.0%	0%

Exhibit F

FAX

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